GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: August 26, 1980

Project Title: New Methods for Biomedical Research

Project No: G-33-D05

Project Director: Dr. Nai-Teng Yu

Sponsor: DHEW/PHS/NIH National Eye Institute (Research Career Development Program)

Agreement Period: From 1 July 1980 Until 30 June 1981 (05 year)

Type Agreement: Grant No. 5-K04-EY00073-05

Amount: $35,805 G-33-D05

Reports Required: Annual Progress Reports with continuation applications; Terminal Progress Report upon Grant expiration.

Sponsor Contact Person (s):

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NOTE: FOLLOW-ON PROJECT TO G-33-D04
(04 year)

Defense Priority Rating: None

Assigned to: Chemistry (School/Laboratory)

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GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

# SPONSORED PROJECT TERMINATION SHEET

Date 12/30/81

**Project Title:** New Methods for Biomedical Research

**Project No:** G-33-D05

**Project Director:** Dr. Nai-Teng Yu

**Sponsor:** DHEW/PHS/NIH National Eye Institute (Research Career Development Program)

**Effective Termination Date:** 6/30/81

**Clearance of Accounting Charges:** 6/30/81

**Grant/Contract Closeout Actions Remaining:**

- [ ] Final Invoice and Closing Documents
- [ ] Final Fiscal Report
- [x] Final Report of Inventions
- [ ] Govt. Property Inventory & Related Certificate
- [ ] Classified Material Certificate
- [ ] Other _____

**Assigned to:** Chemistry (School/Laboratory)

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FORM OCA 10:781

G33-D25

# Final Progress Report

Grant Number: 5K04EY00073
Period Covered: 7/1/76 - 6/30/81
Investigator: Yu, Nai-Teng
Institution: Georgia Institute of Technology
Atlanta, GA 30332
Title of Project: New Methods for Biomedical Research

The most important accomplishment during the period supported by the N.I.H. Research Career Development Award is the development of Raman scattering technique to a stage that one can obtain high quality Raman spectra of the lens from a live animal without injury to the eye. Raman spectroscopy has been shown to be a useful tool in the study of lens structure (Yu and East, 1975; Kuck, East and Yu, 1976; Yu, East, Chang and Kuck, 1977; East, Chang, Yu and Kuck, 1978; Kuck, Yu and Askren, 1981). Recent applications of the Raman probe have led to the discovery of different fluorescent materials in the lens (Kuck and Yu, 1978; Yu, Kuck and Askren, 1979). Of particular interest is one fluorophor with excitation/emission maxima: 647/672 nm (Kuck, Yu and Askren, 1979). This fluorophor presents itself as a precataractous indicator since it does not appear in the normal young lens, but is highly elevated in brunescent cataracts.

We recognized the possible clinical application of our technique and instrumentation, with some refinement. The ultimate goal of this research is the clinical application as a routine diagnostic procedure in ophthalmology.

In a step-wise fashion we proceeded towards obtaining Raman spectra from live animals. Isolated eyes from rabbits were used first in order to work out orientation and alignment details. An arrangement with a 90° scattering angle was chosen for simplicity. Raman spectra from the rabbit lens in situ can be obtained with a 12 mW laser power at 514.5 nm wavelength.

Human eyes (64 yr. old) were tried, but fluorescence interfered with data collection. With modification, the Raman instrumentation can be used for measuring the fluorophor concentration. Only human eyes with the cornea intact were used. Since most corneas are removed for transplant surgery, these eyes were rare and we had the opportunity of working with them only once.

Pentobarbital was used as an anesthetic initially; later, tholazine was added to the protocol as a "pre-op" medication. Rabbits, even under anesthesia, have a propensity towards twitching. Sneezes, pulsations and other irritations would make the eye move out of alignment. This made spectrum-scanning with the conventional single-channel photo-multiplier tube impossible. Spectrum scanning from 200-3600 $cm^{-1}$ took thirty minutes to run and the animal had to be motionless throughout. This problem was remedied with the installation of a Reticon detector (see Fig. 1). This instrument has a multiple photodiode arrays whose

different pixels accumulate signal simultaneously. The data is integrated over time and therefore inconsistencies in the sample scattering are inconsequential. The position of the eye in the laser beam could be quickly realigned with little change in the quality of the spectrum obtained.

A further advantage of this detection system is that a reduced laser power could be used. Fig. 2 shows the data of Mathies and Yu (1978) who compared the quality of spectra obtained from an intact bovine lens using a conventional photomultiplier and a Silicon Intensified Target. They were able to obtain satisfactory spectra with as little as 1 mW laser power using the SIT. This detector, however, does not have the sensitivity of the Reticon we have used in the laser wavelength region. The increased sensitivity of our detector has permitted the aquisition of suitable spectra in as little as 15 seconds, with excellent spectra in 2-1/2 minutes, whereas the spectra of Mathies and Yu required 15 minutes or more.

The only disadvantage of our set-up is that it is designed for high resolution and therefore it has a greater dispersion. Therefore, several "snap-shorts" of the spectrum must be made in order to cover the complete spectrum. Each spectrum consists of 5 pieces that were spliced together.

## Conclusions

The structural information obtainable from the Raman spectrum has been documented, but nowhere in the literature has a spectrum from the lens of a live organism been reported. This accomplishment represents a large step towards the use of this technique in the physician's office.

The difficulties that has yet to be overcome include the dispersion and final design. With a monochromator design for a laser degree of dispersion, only a single "exposure" will be necessary to obtain a spectrum. In the interest of obtaining a fluorescent spectrum from a brunescent cataract, an even shorter exposure time than that needed for Raman spectroscopy will provide a suitable spectrum. This is in part due to the fact that fluorescence is generally more intense than Raman scattering but also due to the "squeezing" effect of the decreased dispersion. Also, fluorescent spectroscopy does not require high resolution.

With increased use of lasers in Medicine, particularly in the field of ophthalmology, only minor modification of current diagnostic and therapeutic instrumentation need to be made before this technique will be ready for clinical investigation. Information about the clinical development (etiology) of brunescent cataracts that this technique could provide will be useful to the ophthalmologist.

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Publications which acknowledge the support by EY00073 Award:

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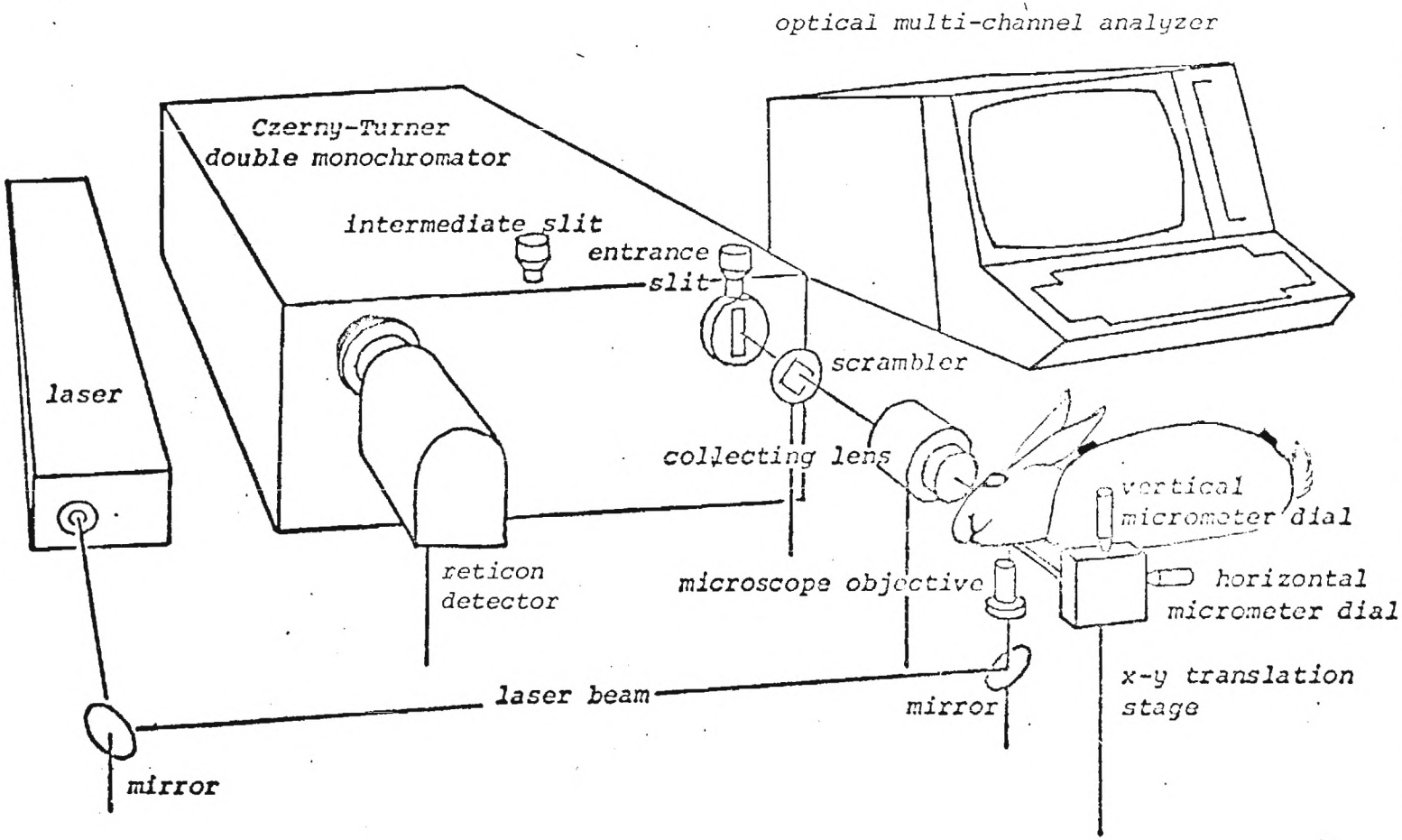
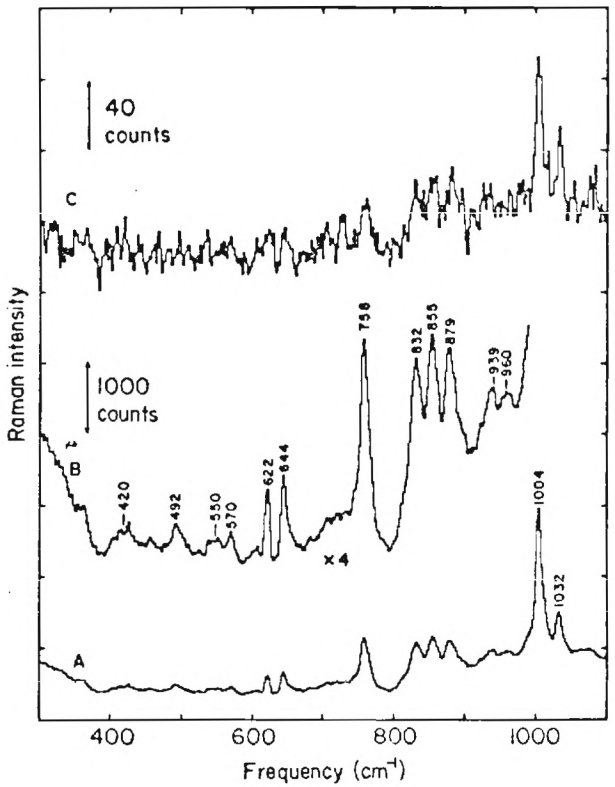
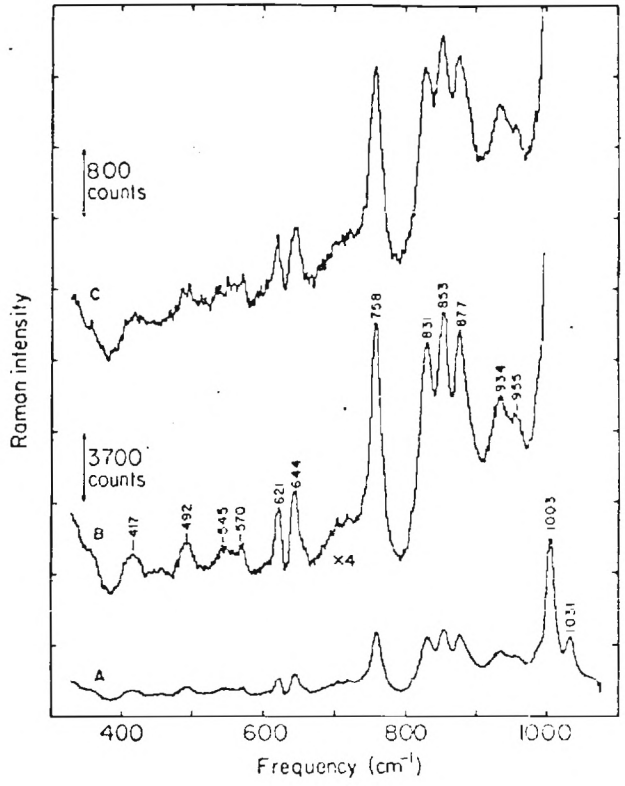



Highly schematic presentation of the experimental instrumentation.

Fig. 1




Raman spectra of low frequency vibrations of bovine lens proteins using PMT–PC detection (A) Spectrum taken with 100 mW 514.5 nm and 5 $cm^{-1}$ (300 $\mu$) slits. Data acquisition time 800 sec. (B) Four-fold expansion of *A*. (C) Spectrum taken with 1 mW 514.5 nm and 6.7 $cm^{-1}$ (400 $\mu$) slits. Data acquisition time 800 sec.




Raman spectra of low frequency vibrations of bovine lens proteins using SIT–OMA detection. (A) Spectrum taken with 100 mW 514.5 nm, no delays, and 10.5 $cm^{-1}$ (150 $\mu$) slits. Total data integration time 29.5 sec (900 cycles). One bin is equal to 1.54 $cm^{-1}$. (B) Four-fold expansion of *A*. (C) Spectrum taken with 1 mW 514.5 nm, 14 $cm^{-1}$ (200 $\mu$) slits, and 100 delay cycles. Total data integration time 655 sec (20,000 cycles).

Fig. 2

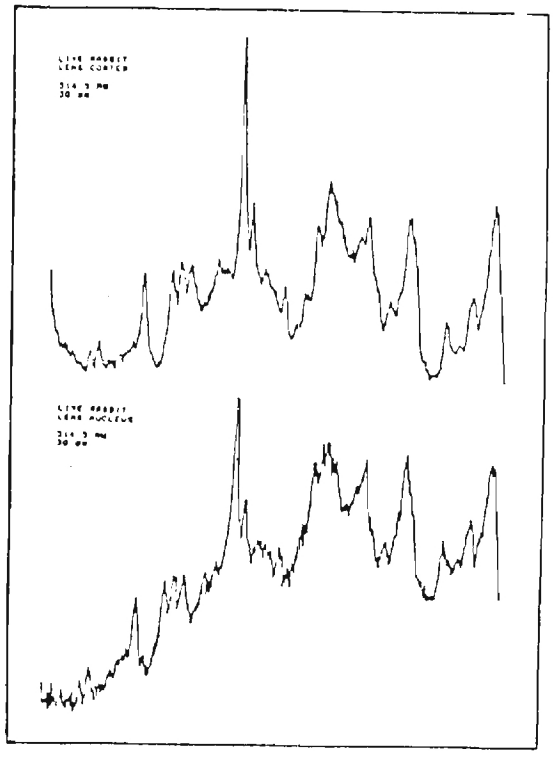


Fig. 3